U. S. NAVAL SUBMARINE MEDICAL CENTER

Submarine Base, Groton, Conn.

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A STUDY OF WHOLE SALIVA AND DENTAL CALCULUS IN SUBMARINERS

by

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Bureau of Medicine and Surgery, Navy Department
Research Work Unit MR005.19-6024.09

Released by:

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SUBMARINE MEDICAL RESEARCH LABORATORY
NAVAL SUBMARINE MEDICAL CENTER REPORT NO. 572

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SUMMARY PAGE

THE PROBLEM

Dental disease, including periodontal disease, has historically been a common health problem on submarine patrols. Dental calculus is considered to play an influential role in periodontal disease. Little has been established about the oral factors which possibly influence rate of calculus formation. Some studies indicate salivary pH may play a role in this formation. Acknowledging the possible role of salivary pH, a study was needed to assess changes in bicarbonate and calcium-phosphorus levels (and subsequent changes in calcium-phosphorus ion product) and their relationships to the dental calculus formation rate.

FINDINGS

Of the different parameters under study, only the elevation of salivary bicarbonate during prolonged exposure to increased levels of atmospheric CO_2 demonstrated statistical significance. An unexpected finding was a remarkably high calculus calcium phosphorus ratio under patrol conditions.

APPLICATIONS

The elevation of salivary bicarbonate and the high calculus calcium phosphorus ratios demonstrated during exposure to high CO_2 atmospheres should encourage further evaluation of these factors on the calculus and surface enamel composition in submariners.

ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Research Work Unit MR005.19-6024 — Effect of Stresses of Submarine Service on Oral Health. This report has been designated as Submarine Medical Research Laboratory Report No. 572. It is Report No. 9 on this Work Unit, and was approved for publication as of 14 March 1969.

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ABSTRACT

Dental calculus is known to be related to the initiation of periodontal disease. Recent studies have indicated that salivary acid-base changes occur on patrol and that such changes may reasonably be expected to influence the formation and composition of dental calculus. A study was completed to examine these factors. Whole saliva samples were collected from eleven crewmen of a Polaris submarine at four periods in the patrol cycle: prepatrol, one week after the patrol started, after the 27th day of patrol, and during the last week of patrol. The calculus formation rate was assessed at the end of this time and a calculus sample was removed for analysis. Analyses revealed a significant increase in the salivary bicarbonate as the patrol progressed and a remarkably high calcium-phosphorus ratio was seen in the calculus samples. All others which were investigated were found to be essentially negative.

A STUDY OF WHOLE SALIVA AND DENTAL CALCULUS IN SUBMARINERS

INTRODUCTION

The close association between the presence of dental calculus and periodontal disease has been acknowledged for some time[1, 2, 3, 4] The exact nature of their relationship is not clearly understood and subject to some controversy. The mechanical irritating action of dental calculus on soft tissue structures has been described in dental texts[5]. An accumulation of harmful bacterial plaque may be encouraged by the irregular surface of the calculus[2]. It has been proposed that the rate of calculus formation is heavily influenced by an elevation of salivary pH, resulting in precipitation calcium and phosphorus complexes into organic matrix on the teeth[6].

Since the oral health of FBM personnel is of vital importance[7] and the nature of FBM patrols provides such a unique opportunity for clinical investigation, a study was initiated to evaluate parameters of the oral environment which may contribute to calculus formation.

MATERIALS AND METHODS

Eleven volunteers from the Gold crew of the USS WILL ROGERS (SSBN 659) participated in the study. Each man prior to embarking on patrol received thorough inspection and cleaning of his lower incisor teeth. Whole saliva samples were collected 30 minutes prior to breakfast, lunch and supper on two successive days at four periods during the patrol cycle: (1) prior to embarking; (2) at one week into patrol; (3) after the 27th day; and, (4) last week of patrol. Two samples were taken at each collection period. The first sample was unstimulated, i.e., collected from whole saliva, as it forms naturally in the mouth. The second sample was stimulated, collected following brief period of mastication of one-half stick of paraffin. Thus, each subject gave forty-eight samples. A volume of 150 microliters was collected from each sample, placed in a plastic microcentrifuge tube and refrigerated.

Every subject was instructed neither to eat, drink nor smoke for 30 minutes prior to a collection period. No other dietary or environmental restrictions were imposed. Subjects were instructed to incline the head forward allowing whole saliva to pool in the mouth, discard pooled saliva twice, and then collect the third sample in paraffin coated cups. A micropipette was used to draw measured samples.

Upon return from patrol each man was reexamined and measurement of dental calculus formation was made[8] and calculus samples were taken for analysis. Whole saliva samples were analyzed by micro techniques for calcium phosphorus and bicarbonate content. Bicarbonate was analyzed by the manometric method of Van Slyke. Calcium was analyzed by a micro-adaptation of the method of Diehl and Ellingboe[9] and phosphorus, by a micro-adaptation of the method of Fiske and Subbarow[10]. The dental calculus samples were ashed on platinum foil at 900°C. The ash was then analyzed in a manner described by Piebenga and Shiller[11].

RESULTS

The calculus analyses are presented in Table I. Unfortunately, with the exception of subject number five, there were no really high calculus formers. The calcium phosphorus (CaP) ratios indicate a high degree of calcification of the calculus, but the values are noted to be quite a bit higher than those previously reported by the Dental Branch, Submarine Medical Research Laboratory. There was a significant degree of correlation between the Ca/P ratio and the amount of calculus formed.

Table I
Calculus Formation

Subject	Formation rate score	Ca/P ratio w/w
1	1.5	2.37
2	3.0	2.24
3	2.5	2.27
4	3.5	2.31
5	10.0	3.62
6	3.0	2.11
7	3.0	2.17
8	1.5	1.81
9	2.0	2.87
10	2.5	2.10
11	0.5	1.68
Mean	3.0 ± 0.75	2.32 ± 0.16*

Correlation (rate vs ratio) $r = +0.84$
*Standard error of the mean.

The whole saliva analyses are presented in Tables II, III and IV. It is noted that the calcium concentrations were rather uniform. Only in the early part of the patrol was the salivary calcium elevated ($P < .05$ based on the critical ratio test). The inorganic phosphorus values were similar to those of calcium except that a depression in phosphorous content was noted in the fifth week of patrol. The most significant findings occurred in the case of salivary bicarbonate (measured as CO_2). There was a progressive increase which became sustained after the fifth week of patrol.

In order to discover overall relationships between the components under study and calculus formation rates the subjects were divided into high and low calculus formers (scores of 2.5 and below and scores of 3.0 and above). Analyses of variance were then performed for the relationship between calculus level and the Ca $\times$ P mQ/100 ml^2 values (Table V) and the bicarbonate levels (Table VI). In neither case did the calculus formation level variable account for a significant degree of the total variance. Similar analyses were performed in the case of the actual calcium and phosphorus levels in relation to the calculus formation rate. No relationships were found.

Table II
Whole Saliva Calcium Content on a FBM Patrol (meq/L)

Collection Period	Calcium content (mean ± standard error of mean)
Prepatrol	2.87 ± 0.166
First week of patrol	3.42 ± 0.184*
Fifth week of patrol	2.92 ± 0.117
Last week of patrol	2.90 ± 0.182
Population	3.05

*Significantly different from population mean (critical ratio test)

Table III
Whole Saliva Inorganic Phosphorus Content on a FBM Patrol (mg/100 ml)

Collection Period	Phosphorus content (mean ± standard error of mean)
Prepatrol	32.71 ± 0.818*
First week of patrol	32.34 ± 0.733*
Fifth week of patrol	28.78 ± 0.515*
Last week of patrol	30.78 ± 0.587
Population	30.89

*Significantly different from population mean (critical ratio test)

Table IV
Whole Saliva Bicarbonate content on a FBM Patrol (meq/L)

Collection Period	HCO_3 content (mean ± standard error of mean)
Prepatrol	7.76 ± 1.09*
First week of patrol	14.08 ± 1.38*
Fifth week of patrol	27.11 ± 1.62*
Last week of patrol	27.92 ± 1.46*
Population	20.31

*Significantly different from population mean (critical ratio test)

DISCUSSION AND CONCLUSIONS

The primary results for which this study was designed, namely the salivary components — calculus formation rate relationships, were essentially negative. Two possible conclusions may be drawn from these findings. One, there may actually be no such relationship; or two, the methods employed

Table V
Relationship Between Salivary Ca × P Ion Products and Calculus Formation on Patrol

Source of Variance	Sum of Squares ss	Degrees of Freedom df	Mean Squares ms	f
Patrol period	13357.10	3	4452.36	*
Calculus formation	446.83	1	446.83	*
Interaction	3715.22	3	1238.4	*
Within groups (error)	225151.97	36	6254.22	
Total	242671.12	44		

*Not statistically significant

Table VI
Relationship Between Salivary Bicarbonate and Calculus Formation on Patrol

Source of Variance	Sum of Squares ss	Degrees of Freedom df	Mean Squares ms	f
Patrol period	3297.78	3	1099.26	20.87**
Calculus formation	44.38	1	44.38	*
Interaction	51.30	3	17.10	*
Within groups (error)	1896.33	36	52.67	
Total	5289.79	44		

*Not statistically significant
**$P < .001$

may have been insufficiently precise. The second possibility deserves additional comment. When one looks at the calculus formation rates, these data are seen to be very closely grouped. Only one subject had a very high formation rate and only one subject had essentially no calculus formed. The remainder showed essentially moderate formation rates. This poor spread of values could easily have prohibited the appearance of the presumed relationships. Another factor under this second possible explanation is the rather long term collections of calculus for the formation rate assessment. This time period was three months and encompassed the last two weeks of the prepatrol period, the entire patrol and a short time of the post patrol period. A much shorter collection time would certainly afford better chances of demonstrating the fine relationship sought. Parenthetically, another submarine medical officer is currently investigating the feasibility of using another calculus index which employs a two week collection period.

One unexpected finding in this study was the large increase in the whole saliva CO_2 as the patrol progressed. Hughes[12] had demonstrated a modest, but not statistically significant, increase in the CO_2 content of the pure parotid secretion on patrol. The present results should certainly be considered of greater practical importance, since the fluids involved are those which actually bathe the teeth. The reasons for these unusual findings in the present study cannot be stated with finality; however, a plausible explanation could be based on the oral fluid — ambient atmosphere exchange differences which may occur with changes in the FBM atmosphere.

The data in Table I demonstrates a significant positive correlation between the calcium phosphorus ratio and the amount of calculus formed. This was not surprising, since one would expect the larger deposits to contain proportionately less salivary sediments. The calcium phosphorus ratios certainly indicate a high degree of calcification.

In fact this very high ratio points to another remarkable difference between these findings and previously reported ones. The current concept of calculus formation supposes that the primary mechanism is a precipitation of calcium phosphate and to a much lesser degree calcium carbonate and other phosphates. A calculus formation of pure $Ca_3(PO_4)_2$ would yield a Ca/P ratio of two on a weight/weight basis. Actually, calculus collected in the Antarctic[13] and from ordinary subjects[14] yield Ca/P ratios much lower than this. The greatly increased ratios in the present study would tend to lead to the possibility of a changed calculus and surface enamel composition with the changed atmosphere on an FBM patrol. One can seriously consider the role of carbonates to explain this high Ca/P ratio. Actual analyses for carbonates in calculus and in the surface enamel would be justified and possibly indicated on the basis of these findings.

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Dental calculus is known to be related to the initiation of periodontal disease. Recent studies have indicated that salivary acid-base changes occur on patrol and that such changes may reasonably be expected to influence the formation and composition of dental calculus. A study was completed to examine these factors. Whole saliva samples were collected from 11 crewmen of a Polaris submarine at four periods in the patrol cycle: prepatrol, one week after the patrol started, after the 27th day of patrol, and during the last week of patrol. The calculus formation rate was assessed at the end of this time and a calculus sample was removed for analysis. Analyses revealed a significant increase in the salivary bicarbonate as the patrol progressed and a remarkably high calcium-phosphorus ratio was seen in the calculus samples. All other sought relationships were essentially negative.

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